
HOW WELL DOES CAMELINA TOLERATE ROOT ZONE SALINITY?

H. Steppuhn¹, K.G. Wall, K.C. Falk², R. Zhou² and S.K. Brandt

¹ *Semiarid Prairie Agricultural Research Centre, Agriculture & Agri-Food Canada,
P.O Box 1030, Swift Current, Saskatchewan S9H 3X2*

² *Saskatoon Research Centre, Agriculture and Agri-Food Canada,
107 Science Place, Saskatoon, Saskatchewan S7N 0X2*

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Abstract

Crops of CS15 camelina and InVigor 9590 canola grown in saline rooting media were evaluated for plant emergence, height growth, grain yield, and oil production. The experiment utilized Canada's Salinity Tolerance Testing Facility featuring a controlled environment simulating field conditions. Test plants were grown in sand tanks flushed four times daily with hydroponic solutions consisting of nutrients and salts dominated by sulphate anions targeting electrical conductivity (EC_{sol}) treatments of 1.4 (nutrients only), 3, 6, 10, 15, 21, and 28 dS m⁻¹. A comparison of the mid-salinity tolerance indices for the two crops indicated that the camelina registered about half of that for the canola. This difference narrowed for the salinity at the slight and severe ends of the total range.

Introduction

Camelina sativa L., a new oilseed crop, is targeted for cultivation across the semiarid Brown and Dark Brown soils of western Canada. Until recently, camelina had only rarely been cultivated in North America, although it had been known, studied, recommended, and touted as a potentially valuable crop. Currently, considerable interest in camelina stems from its potential to serve as feedstock for biodiesel fuel production in cool, semiarid climates. Also, today's seeding implements tend to better cope with the very small size of camelina seed (0.9-1.5 g per 1000 seed). The crop appears to be adapted to dryer environments and may serve to replace biodiesel canola in the Brown Soil Zone. Selected cultivars of canola are currently recommended for the millions of hectares in fields containing slightly and moderately saline soils on the Prairies; how does camelina compare to canola in salinity tolerance?

Camelina seed oil and meal test rich in linolenic and other OMEGA acids, and generally contain fatty acid compositions which rank largely as unsaturated. The seed meal registers low in glucosinolates and features a favourable balance of amino acids, making it valuable as a

livestock feed.

In the experiment reported here, emergence, shoot height, grain yield, and oil content were evaluated under the controlled conditions of Canada's Salt Tolerance Testing Facility at Swift Current, Saskatchewan. The results cover a full range of sulphate-based, hydroponic rooting solutions (from negligibly- through severely-saline). The objective of this study is to compare the inherent salinity tolerance of CS15 camelina to that of InVigor 9590 canola for crops grown subjected to hydroponically-salinized root zones.

Materials and Methods

Test Seed Bayer CropScience provided the InVigor 9590 canola seed used in this experiment. This cultivar falls into the Oilseed Spring Hybrid Class and contains the novel Liberty-Link gene for herbicide resistance. The CS15 camelina seed utilized in this study originated from breeder supplies at the Saskatoon Research Centre of Agriculture and Agri-Food Canada. This genotype registered among the best in seed production in previous field trials (Gugel and Falk 2006).

Testing The experiment was conducted in a greenhouse featuring a controlled environment using hydroponically-nourished sand tanks. This testing facility, located at Swift Current, Saskatchewan, features automatic control over irrigation, fertility, seedbed and root-zone salinity, and ambient temperature integrated over time under an electronic, programmable logic controller (Steppuhn and Wall 1999). Plastic grow tanks (cylinders 0.85 m dia. x 1.0 m deep) were used which contain washed silica sand (99.8% pure) having an average bulk density of 1.65 Mg m^{-3} and a sand-surface area of 0.57 m^2 . At saturation, the sand uniformly holds water at a volumetric content of 31.3%.

The seedbeds and root zones were flushed four times daily (01:00, 09:00, 13:00, and 17:00 hour) with aqueous solutions containing modified Hoagland nutrients consisting of $\text{Ca}(\text{NO}_3)_2$, KNO_3 , KH_2PO_4 , MgSO_4 , chelated Fe, NH_4NO_3 , KCl, H_3BO_4 , plus trace elements including Mn, Zn, Cu, Si, and Mo (Hoagland and Arnon 1950). Fortified with these nutrients, seven different treatment solutions were prepared by adding proportionate quantities of CaCl_2 , NaCl, MgSO_4 , and Na_2SO_4 sufficiently to obtain solutions with electrical conductivities targeted to equal 1.4, 3, 6, 10, 15, 20, and 28 dS m^{-1} . These test solutions represent salinity levels from negligible (nutrients-only) to severely saline (United States Salinity Laboratory Staff 1954). All nutrients and salt complements were prepared and added to the test solutions prior to seeding. The pH-values of the test solutions averaged 7.8 across all the treatments.

Each flushing (irrigation) supplied treatment solutions to the sand tanks for five minutes, which completely saturated the sand followed by time for the sand to drain to field capacity. The drained solutions returned to 612-L supply reservoirs, where they were held ready for the next flushing. The electrical conductivities of the irrigated solutions were checked initially, weekly, and at harvest, and assumed equal to the solutions in contact with the seed and roots (EC_{sol}). Water lost by evapotranspiration was replenished weekly or when necessary to maintain the concentrations of salts in solution. The actual EC_{sol} averaged 1.36, 2.98, 6.05, 10.00, 14.67,

19.92, and 27.02 dS m⁻¹ during the course of the experiment. Soil solution electrical conductivities (EC_{sol}) in dS m⁻¹ relate to equivalent electrical conductivity of saturated soil paste extracts (EC_e) in dS m⁻¹, as detailed in Ayers and Westcot (1985), by the approximate relationship:

$$EC_e \approx 0.5(EC_{sol}) \quad [1]$$

The test was conducted with an appropriate time course for day/night sequences (adjusted every four days) mimicking an April 27th seeding date at 50° north latitude. Supplemental lighting from 475-W sodium lamps positioned 1.5 m above the sand surfaces extend day-lengths. Lamps were strategically positioned overhead in order to obtain measured radiation intensities averaging 7.9 kJ m⁻² min⁻¹ with a uniformity coefficient of 0.9 across the entire test facility. Temperature setpoints were automatically reset hourly according to a 24-hour diurnal schedule and ranged from 14 to 24°C with ambient temperatures maintained within one or two degrees of the setpoints.

The tank arrangements followed a randomized block design with respect to the test crops and salinity levels, modified slightly to eliminate any bias caused by the taller plants blocking solar radiation associated with low sun angles. In these tests, full complements of salts were added to the nutrient water supplies prior to seeding. One hundred-four seed from each of the two test crops were sown 13 mm deep into the sand in rows spaced 152 mm apart within each sand tank (182.5 seed m⁻²). Upon completion of emergence (after 32 days), the remaining plants were subsequently thinned to 64 plants per tank (112 plants m⁻²).

Measurements and Analyses

Within each treatment, the response of the plants to root-zone salinity was determined by measuring emergence, plant height, oven-dried grain (seed) yield, and oil content. Measurements from each test crop were averaged and related to electrical conductivities of the test solutions (EC_{sol}).

Plant Emergence and Survival Two flushes with the test solutions preceded seeding in order to firm the seedbed, and a template guided placement of each seed into a known position within each seedbed. This allowed assessment of the plant emergence and survival associated for each planted seed on a daily basis. Any protrusion of the plant above the sand surface counted it as emerged. Records were kept on electronic copies of the seeding template. This practice resulted in daily counts per tank of the number of newly emerged plants and their survival with time.

Plant Height Plant height served to compare plant growth among the treatments and was determined from repeated weekly measurements of the same ten plants per tank. The seed in the experiment were planted on September 27th and growth measured on Oct 23, 30, Nov 6, 13, 20, 27, Dec 4, 11, 18, Jan 8 (camelina harvested), and 18 (3 days before the canola harvest). These dates mark the number of days after seeding for the growth measurements: 26, 33, 40, 47, 54, 61, 68, 75, 82, and 103 (camelina) or 113 days (canola). The plant height data at harvest were

analyzed with an analysis-of-variance and compared for effects of salinity level and crop (SAS 2007).

Grain Yield The above-ground portion of each test plant was cut when the crop would normally have been swathed, and the harvested shoot material from each tank placed in a separate cloth bag and oven-dried at 35 °C. After drying, the contents of the bags were massed, and the grain threshed, massed and collated according to treatment. Dividing these masses per tank by 0.57 m² resulted in shoot and grain yields expressed in g m⁻². The yields from the replicate grow tanks per treatment were reported as averages. To standardize the production obtained under the salinity treatments, grain yields were also expressed on a relative basis. The usual procedure for converting absolute yield (Y) to relative yield (Y_r) employs a scaling divisor (Y_m) equal to the production where salinity has very little or no influence on the yield (Maas 1990):

$$Y_r = \frac{Y}{Y_m} \quad [2]$$

The Y_m divisor normalizes the data-set and, for non-halophytes, usually equals the maximum yield associated with each treatment expressed in percent.

Various empirical equations have been applied or suggested for describing Y_r as a function of a variable which reflects the average root-zone salinity (C). The measure for C in this study is EC_{sol}, where EC_{sol} equals the electrical conductivity of the test solution in dS m⁻¹. The most recent empirical analog function for determining relative product yield (Y_r) in response to increasing root zone salinity is the modified discount equation (Steppuhn et al. 2005a):

$$Y_r = \frac{1}{1+(C/C_{50})^{\exp(sC_{50})}} \quad [3]$$

where C₅₀ defines C at Y_r = 0.5, and s represents the response curve steepness. The steepness parameter equals the average absolute value of the slope (dY_r dC⁻¹) of the equation through C₅₀ and its steepest segments on either side of C₅₀, evaluated in our study from Y_r = 0.3 to 0.7. The argument sC₅₀ of the exponent in Eq. 3 contributes to a symmetrical convex-concave yield response with the inflection point at C₅₀. The parameter s describes the average unit decrease in relative product yield with unit increase in root-zone salinity.

A single-value index of crop tolerance to root-zone salinity has proved useful for comparing the salinity tolerance of agricultural crops (Steppuhn et al. 2005a). If C₅₀ were enhanced by a term which dictates the shape of the yield response for salinity levels approaching C₅₀, such as the argument of the exponent in Eq. 3, a comprehensive, single-value, Salinity Tolerance Index or ST Index results:

$$ST\ Index = C_{50} (1 + s) \quad [4]$$

where C_{50} and s can be computed as regression constants, or approximated by a visual inspection of the response data.

The grain yield measurements, scaled by the results obtained in the low or salt-free treatments, facilitated comparisons. The scaling divisors for the yield data were determined by substituting Y/Y_m of Eq. 2 into Eq. 3 and solving for Y_m using nonlinear regression software from SAS (2007), which is based on the maximum neighbourhood method of Marquardt (1963) and an optimum interpolation between the Taylor series method and the method of steepest descent (Bates and Watts 1988). These yield data were tested and accepted for homogeneity of variance among means using the Brown-Forsythe, Bartlett, and Welch tests (SAS 2007).

Determined for each test crop grown under each salinity treatment, the relative grain yield, regress-fitted to the discount response function (Eq. 3), resulted in separate response functions, one per crop. From these functions, respective C_{50} and s values were derived for each crop using the same nonlinear software as before (SAS 2007). These parameters led to Salinity Tolerance Indices based on Eq. 4 indicating the relative salinity tolerances between the two crops. A statistical covariance procedure utilizing paired t-tests served to compare the discount response functions for similarity and differences among the test crops. These comparisons provided the basis for assigning differences in relative salinity tolerances for the two crops.

Seed Oil Content Samples of the harvested grain seed from each crop were analyzed for oil content in percent by mass at the Oilseed Laboratory of the Saskatoon Research Centre, Agriculture and Agri-Food Canada (AAFC). The camelina crop failed to produce any seed at the 27.02 dS m^{-1} salinity level.

Results and Discussion

Plant Emergence and Survival Before seeding, the germination test results with seedlots for the two crops averaged 95.0% and 87.0% for the canola and camelina, respectively (data not shown). From 104 seed planted per tank in the experiment, the number which germinated, emerged, and survived as seedlings in the negligible salinity environment (1.36 dS m^{-1}) averaged some 103 canola and 95 camelina plants or 99.0% and 91.3%, respectively. From these maxima, the percentages ranged mostly downward to 76.9% and 13.9% for the canola and camelina respectively under the severe salinity of 27.03 dS m^{-1} (Figure 1). Among the seed planted in the 1.36 , 2.98 , 6.05 , and 10.00 dS m^{-1} tanks, differences in emergence between the two crops tended to be narrow with only very slight, if any, advantage to either crop. At 14.67 , 19.92 , and 27.02 dS m^{-1} , the cumulative number of plants which emerged and survived became progressively less for the camelina compared to the canola. When the maximum number of seedlings emerging and surviving from each crop at the negligible or low salinity level was used to scale the data, the emergence for each crop and salinity treatment was not statistically different until the salinity reached severe (Table 1).

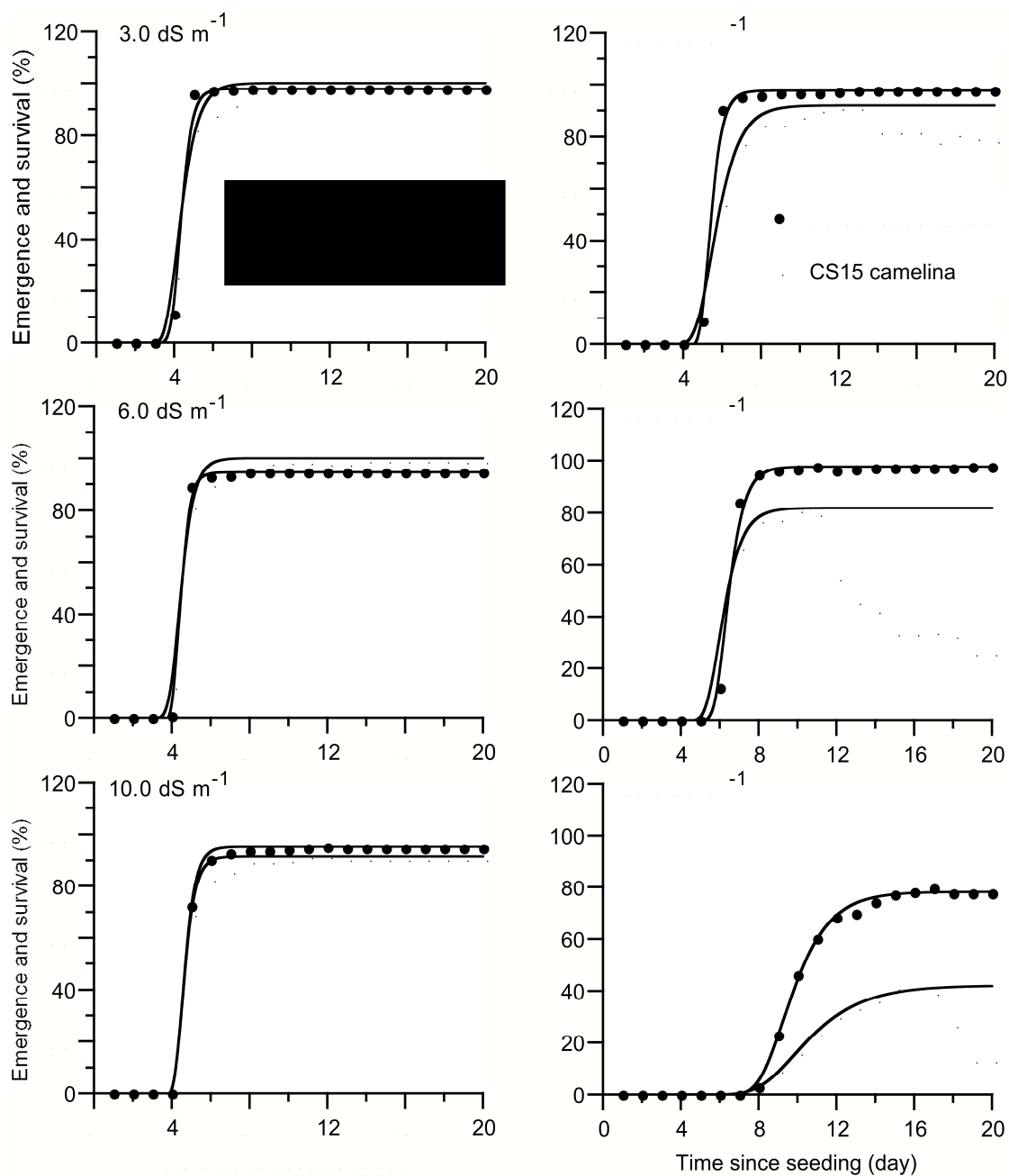


Figure 1. Average cumulative emergence and survival of InVigor 9590 and CS15 Camelina plants (in percent of the best experimental total among all salinity levels) subjected to rooting substrates averaging 3.0, 6.0, 10.0, 14.7, 19.9, and 27.0 dS m^{-1} .

Table 1. Emergence Values for Each Crop and Salinity Treatment, and Statistical Probability of Obtaining a Greater F-value.

Treatment ^z EC _{sol} ..	Crop and Emergence ^y			
	Canola	Camelina		
dS m ⁻¹	%	%		
1.36	100.0a	91.9a		
2.98	97.7a	100.0a		
6.05	94.5a	100.0a		
10.0	95.2a	92.4a	Probability > F	
14.67	97.6a	91.0a	EC _{sol}	0.0043
19.92	97.6a	81.8b	Crop	0.1201
27.02	79.6b	42.1c	Replicates	0.1270

^z EC_{sol} is the average electric conductivity of the test solution.

^y values followed by the same letter do not differ significantly at $P_v \leq 0.05$ according to the paired means student's t-test.

The maximum cumulated emergence of seedlings growing subjected to the sulphate rooting solutions failed to differ statistically between the camelina and the canola crops until the salinity level reached severe, i.e., 19.92 dS m⁻¹ or greater (Table 1). This leads to the inference that the number of emerged plants which remain viable and grow, albeit slowly under severe salinity, serves as a useful initial indicator for the crop tolerance of saline root-zones. Seedlings, which barely survive in controlled sand tanks, will most likely succumb to disease or insects in field environments. As the growing season progressed, the number of seedlings surviving beyond the time of peak emergence was not sustained for the camelina in either of the two severely saline environments (19.92 and 27.02 dS m⁻¹); in contrast with the canola, the emerged camelina plants tended to died with time.

Plant Height The negative effect of root-zone salinity on average crop growth in height is evident from the measurements obtained over the growth period from day 26 after seeding to harvest (Figure 2). Although the height response curves for the growth of the two crops follow similar shapes, the spread between salt-level responses for the camelina tended to exceed those for the canola. These differences between crops increased with salinity level until the camelina plants at 27.02 dS m⁻¹ all died. The time course for the camelina height appeared proportionately congruent with that for the canola in the three lowest salinity levels, but increasingly lagged the canola trace in the four highest levels. The average height of the canola at any time and at any salinity level tended to exceed that measured in the camelina. Average plant heights at the time of respective harvests for the two crops statistically differed by salinity level (probability of a greater F-value < 0.0001) and by crop ($P_v > F$ -value equals 0.0445). Also, the variances for the height data were calculated to be statistically homogeneous.

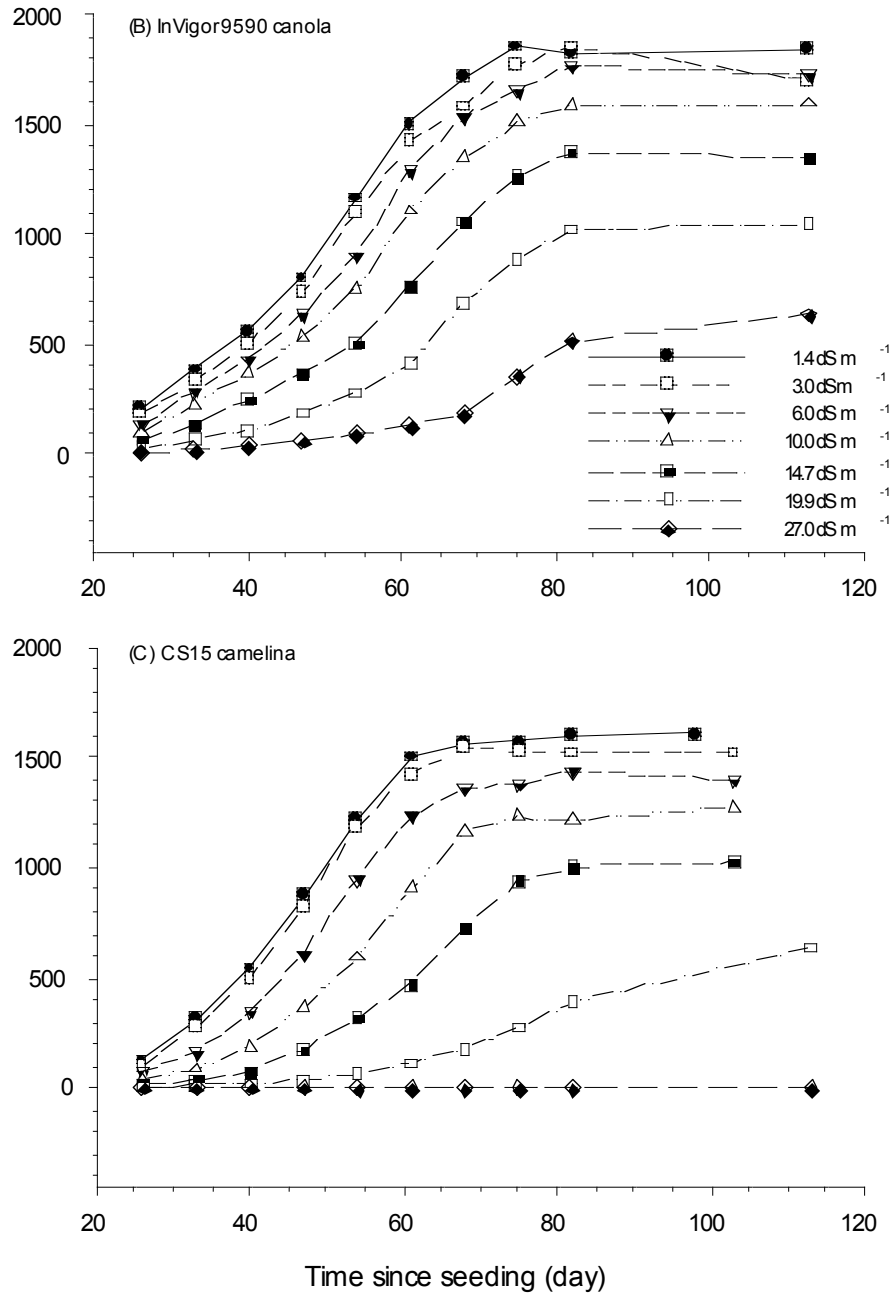


Figure 2. Average plant height of InVigor 9590 canola *Brassica* (B) and CS15 camelina *Camelina* (C) measured more-or-less weekly since seeding for each of seven salinity levels.

Grain Yield The negative impact of persistent root-zone salinity on oven-dried, oilseed yield of the camelina and canola is observed in the harvests taken 103 and 121 days after seeding, respectively (Table 2). Within each crop, seed (grain) yield and shoot biomass tended to decrease as salinity increased. The harvest indices (grain yield/shoot biomass) calculated for the weakest five salinity treatments varied by 3.5% in the canola and 16.2% in the camelina (data not shown).

Table 2. Mean, Oven-dried, Grain (Seed) Yield and Shoot Biomass from InVigor 9590 Canola and CS15 Camelina Crops Grown in Respective Saline Rooting Media Listed by Average Electrical Conductivity of the Test Solution.

Solution ^z EC _{sol}	Canola		Camelina	
	Seed (se) ^y	Shoot (se) ^y	Seed (se) ^y	Shoot (se) ^y
dS m ⁻¹	g m ⁻²	g m ⁻²	g m ⁻²	g m ⁻²
1.36	254.9 (14.0)	1043 (33.4)	144.5 (3.0)	572 (40.5)
2.98	241.9 (7.5)	963 (29.6)	113.2 (14.9)	507 (57.9)
6.05	240.3 (2.7)	949 (17.1)	78.3 (8.7)	370 (51.6)
10.00	199.8 (43.5)	813 (100.8)	71.9 (11.3)	303 (71.6)
14.67	144.8 (4.4)	572 (26.6)	31.4 (9.6)	129 (32.9)
19.92	121.1 (3.1)	414 (5.12)	1.44 (0.20)	9.65 (2.77)
27.02	23.78 (0.32)	162 (29.5)	0	0

^z EC_{sol} equals the average electrical conductivity of the test solution.

^y se equals the standard error.

Conversion of the absolute grain yields (Y) to relative yields (Y_r) indicated less salinity tolerance for the camelina than for the canola at all EC_{sol}-levels (Figure 3). The average absolute grain yield for the InVigor canola averaged close to twice that for the CS15 camelina, perhaps because the well-watered crops in this study allowed the hybrid-genetic, production potential of the canola to be amply expressed.

Regression fits of the modified discount equation (Eq. 3) for relative oilseed yield plotted as a function of root-zone salinity resulted in respective least-square r² and root mean square error values of 0.944 and 0.0674 for the canola and 0.916 and 0.1112 for the camelina (Figure 3). The resulting C₅₀-values (EC_{sol}-based) equalled 16.9 and 6.8 dS m⁻¹ for the InVigor 9590 canola and the CS15 camelina, respectively (Table 3). With Eq. 1, the mean C₅₀-value (EC_{sol}-based) reported for dryland canola by Steppuhn et al. (2005b) is 14.2 dS m⁻¹, or a difference of 2.7 dS m⁻¹ less than that measured with the InVigor 9590 canola in the study presented herein.

The salinity tolerance index (STI), derived from Eq. 4, indicates that the respective salinity tolerance based on a STI difference of 10.65 dS m⁻¹ placed the canola well over that of the camelina (Table 3). According to Steppuhn et al. (2005b), the average STI for dryland canola registers 16.00 dS m⁻¹ (EC_{sol}-equivalent), some 2.0 dS m⁻¹ less than measured for InVigor 9590 in this experiment. In a comparative trial with barley, the STI derived for an earlier InVigor

(2573) equalled 16.7 dS m^{-1} , or 1.3 dS m^{-1} less than the InVigor (9590) in this study (Steppuhn and Raney 2005). One explanation is that salinity tolerance of the InVigor breeding line has improved as the genotype improved.

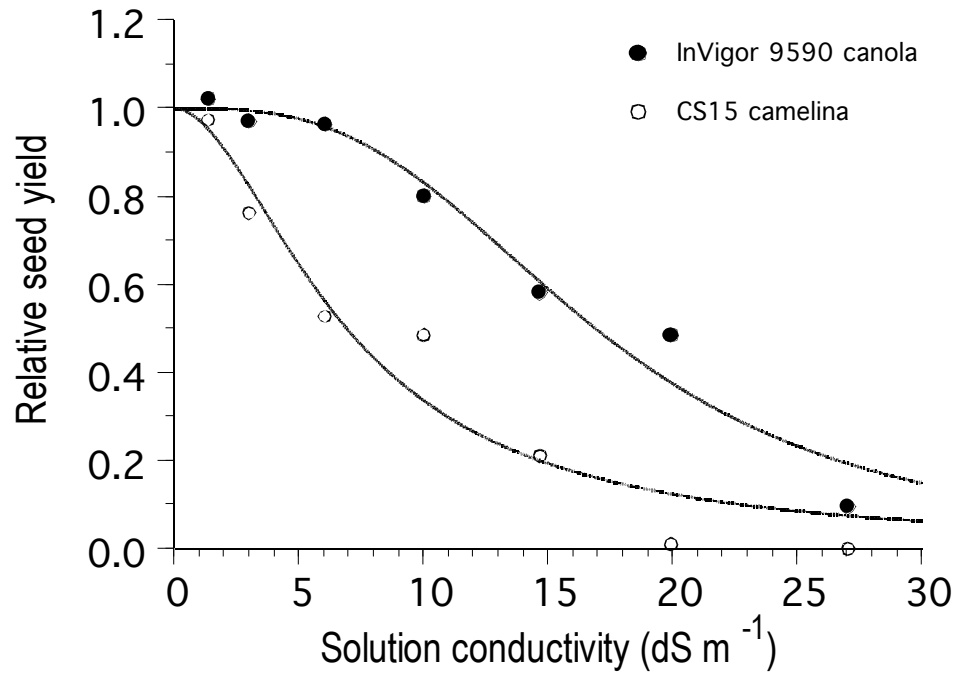


Figure 3. Mean relative seed yield for InVigor 9590 canola and CS15 camelina crops grown in increasingly saline root zones fitted to the discount function [Eq. 3].

Table 3. Response Function Parameters and Salinity Tolerance Index (STI) for Relative Grain (Seed) Yield (Y_r) with Standard Error in Parenthesis for InVigor 9590 Canola and CS15 Camelina Crops Grown in Sulphate-based Saline Media.

Crop	N^y	Parameter & Salinity Tolerance Index (STI) ^z			
		Y_m^x	$C_{50} \text{ (se)}^w$	$s \text{ (se)}^w$	STI
		g m^{-2}	dS m^{-1}		dS m^{-1}
Canola	18	249	16.91 (0.76)	0.0658 (0.0089)	18.02
Camelina	18	148	6.78 (0.64)	0.0868 (0.0235)	7.37

^z The Salinity Tolerance Index = $C_{50} + sC_{50}$ which is derived from the discount response function [Eq. 7]: $Y_r = 1 / [1 + (C/C_{50})^{\exp(s C_{50})}]$, where $C = EC_{\text{sol}}$ and C_{50} defines C at $Y_r = 0.5$, and s represents the response curve steepness.

^y N equals the number of samples.

^x Y_m equals seed yield where salinity has little or no influence.

^w se equals the standard error.

The covariance t-test analyses of the relative grain (seed) yields across the full range of salinity treatments used two sets of Eq. 4 parameters for each crop. The first set was derived from nonlinear regressions in fitting the discount response equation to the grain yield-dS m⁻¹ data resulted in the fitted parameters, C₅₀ and s, obtained for each crop (Table 3). The second set for each crop came from the other crop, i.e., that for the canola, from the camelina, and that for the camelina from the canola (Table 4). To obtain statistical inferences, the relative responses of grain yields for each crop were analysed using paired covariance t-tests with each of the C₅₀ and s parameters. Results from these covariance analyses indicate that the discount response functions for the InVigor 9590 canola or for the CS15 camelina statistically could not be used interchangeably with each other; they were statistically different with α -probabilities for a greater t-value exceeding 0.05.

Table 4. Statistics from Four Separate Covariance Analyses (by paired t-tests) between Measured Relative Grain (Seed) Yield and Respective Discount Response Functions for Comparing InVigor 9590 Canola and CS15 Camelina Crops Grown in Seven Saline Rooting Solutions from Negligibly through Severely Saline.

<u>Crop & measured yield statistic</u>	<u>Mean measured relative Yield</u>	<u>Fitted discount response function^z</u>	
		Canola	Camelina
<u>InVigor 9590 canola</u>	0.711		
Covariance:			
Mean difference		0.0029	-0.1601
Standard error		0.0176	0.0418
Prob.> t ^y		0.8694	<0.0001 ^{**}
Degrees of freedom		17	17
 <u>CS15 camelina</u>	 0.520		
Covariance:			
Mean difference		0.2610	0.0097
Standard error		0.0404	0.0257
Prob.> t ^y		<0.0001 ^{**}	0.7113
Degrees of freedom		17	17

^z The computed relative yield (Y_r) values and statistics from Eq. 7 ($Y_r = 1 / [1 + (C/C_{50})^{\exp(s C_{50})}]$) using seven salinity levels (C) and C₅₀ & s as function parameters from statistical fits resulting from nonlinear regressions with measured data from each genotype.

^y The Prob.>|t| equals the probability for a greater absolute t-value where ^{**} signals computed and measured values which are significantly different with a Type I error probability < 0.010.

Seed Oil Content The percentage of oil contained in the oilseed harvested from plants grown in saline rooting media remained relatively constant until 20 and 10 dS m⁻¹ for the InVigor 9590 canola and the CS15 camelina, respectively (Figure 4). The initial plateau in oil content for the canola as salinity increased agreed with a similar observation in an earlier study (Steppuhn and Rainey 2005). Oil content in oilseed derived from both oilseed plants subjected to root-zone salinity, even those grown in severe salinity, still yielded percentages greater than 30% in both crops.

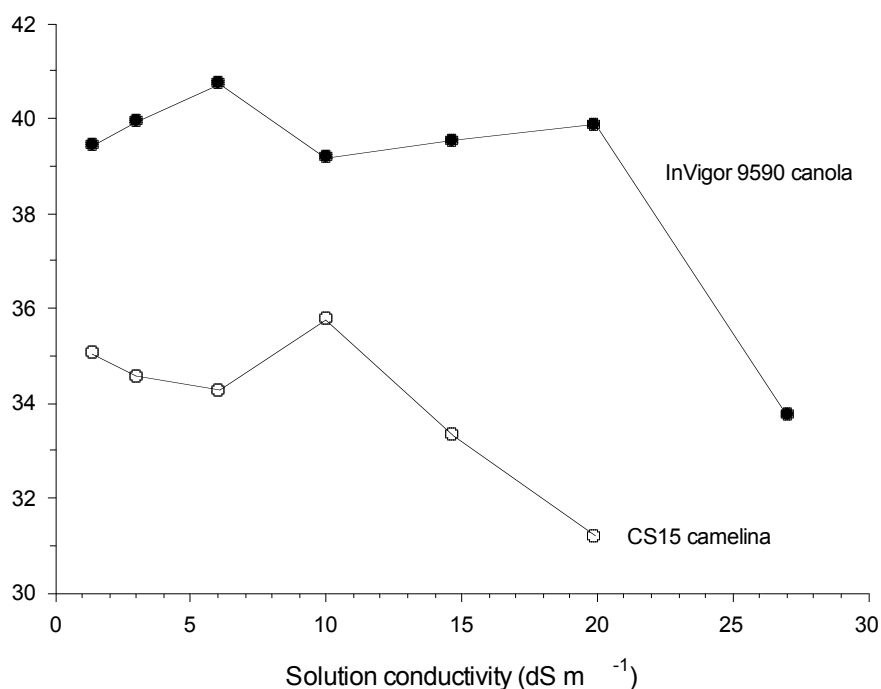


Figure 4. Mean concentration of oil in CS15 camelina and InVigor 9590 canola oilseed (% by mass) related to solution conductivity (dS m⁻¹) of the rooting medium.

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